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EFFECT OF X RAYS AND
 ^{60}Co GAMMA RAYS ON THE
LIVER ENZYME SYSTEM RESPONSIBLE
FOR FATTY ACID SYNTHESIS

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EFFECT OF X RAYS AND ^{60}Co GAMMA RAYS ON THE LIVER ENZYME
SYSTEM RESPONSIBLE FOR FATTY ACID SYNTHESIS

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FOREWORD
(Nontechnical summary)

The effects of ionizing radiation on the mammalian organism have been studied extensively at the organ and cellular levels and there is considerable information available in the literature on biological events such as replication failure and cell death. However, very little is known about molecular lesions which are the direct cause of cellular dysfunction following irradiation.

Since a relatively small number of ionizing events have far-reaching consequences on the survival of the cell, one can assume that the biologically important macromolecular constituents of the cell, essential to its life, are the major targets of importance. These include proteins (especially enzymes), nucleic acids, and the structural components of the cell such as cellular membranes and complex lipids and complex polysaccharides.

Enzymes are proteins which have "active sites" on their molecules. They are extremely powerful and highly specific catalysts whose presence is necessary for the initiation and performance of the different metabolic processes in the living organism.

Since the liver is the organ in which many of the macromolecules, essential to the life and well-being of animals, are synthesized and broken down, it was thought appropriate to study the effects of ionizing radiation on macromolecules at their site of formation.

The results, which are presented in this report, show that under the described experimental conditions both x rays and ^{60}Co gamma rays greatly stimulate the activity of the liver enzyme system. It was also found that the activities of both the

mitochondrial enzymes and the enzymes present in the particle-free soluble supernatant are affected by irradiation. Furthermore, the activity of the soluble enzymes is stimulated to a much greater extent than that of the mitochondrial enzymes.

ABSTRACT

This report describes effects of in vivo exposure to x rays and ^{60}Co gamma rays on the fatty acid synthesizing liver enzyme system. Both fed and fasted young female Sprague-Dawley rats were utilized in these studies. All irradiated animals received a single whole-body exposure of 1200 R at 20 R/min. The irradiated animals as well as sham irradiated controls were sacrificed at predetermined times after exposure and cell-free liver homogenates which contained the enzyme system under investigation were prepared. It was found that the activity of the liver enzyme system responsible for the biosynthesis of fatty acids is greatly stimulated by x or ^{60}Co gamma rays and that the cytoplasmic soluble enzymes are affected by radiation to a much greater extent than the mitochondrial enzymes.

Blood glucose determinations were carried out in irradiated and control rats as well as in rats which were made alloxan diabetic. The results indicate that the enhancement of enzyme activity observed is not due to an increase in the blood glucose level of the animal as a result of exposure to radiation but to some other factor the nature of which has not yet been elucidated.

I. INTRODUCTION

Although there is considerable information available concerning biological events such as cellular dysfunction and cell death, the exact lesions of initial radiation injury have not yet been precisely identified.

From in vitro experiments considerable evidence exists that ionizing radiations lead to chemical modifications of biologically important macromolecular constituents of the living cell and to disorders in their secondary and tertiary structures.^{2, 6, 15} Fragmentation of the molecules or formation of aggregates may also take place.¹⁴ Furthermore, it has been shown that the activity of enzymes is altered by exposing them in aqueous solutions¹¹ or in the dry state¹² to ionizing radiations, and evidence has been presented that in some instances the molecular lesions are reparable.¹

Changes in the stability of liver mitochondria have been reported in x irradiated mice. It has been found that the in vitro incorporation of acetate into fatty acids and cholesterol by rat liver preparations is greatly affected by exposure of the animals to x rays at doses of 750 R and 2400 R, respectively.^{4, 7} More recently it has been demonstrated that significant cytological changes occur in the liver with some concomitant deterioration of its biological functions postirradiation.^{5, 13}

The objective of this study is to investigate the in vivo effects of ionizing radiations on the activity of the enzyme system which is responsible for the biosynthesis of fatty acids in the liver of rats.

II. PROCEDURES

A total number of 540 female Sprague-Dawley rats weighing 90 to 110 grams were used in this study. Of this number of animals, 432 were utilized to determine

the effect of ionizing radiation on the fatty acid synthesizing liver enzyme system, 60 were used to study the effect of radiation on the blood glucose level, and 48 were used for the experiments designed to localize the intracellular site of the radiation effects. One-half of this last group of animals was utilized to determine the role which microsomes may play in the observed enzymic activity changes. All animals were kept on a high carbohydrate fat-free diet for at least 7 days prior to irradiation.

Fifty-four rats were used for each of eight experiments in the study of the fatty acid synthesizing liver enzyme system. The animals used in each experiment were divided into three groups, as follows: 18 rats were fed up to approximately 3 hours before irradiation (fed rats); 18 rats were starved for 24 hours prior to irradiation (starved rats); and 18 rats were sham irradiated and used as controls (control rats). Food deprivation, before irradiation, was used in order to investigate the possibility of standardizing the nutritional condition of the experimental animals. No food was given to the first two groups of rats following irradiation; however, all animals had free access to water. The third group of animals were fed up to approximately 3 hours prior to the experiment and had free access to water.

The experimental animals were exposed to either x rays or ^{60}Co gamma rays. Four repeated experiments were performed using x rays and four using ^{60}Co gamma rays. The physical characteristics of the exposure sources were as follows:

X rays: A 250 kVp x-ray generator with inherent filtration of 1.2 mm beryllium and with added filtration of 0.95 mm copper was used. The distance from the source to the midline of each rat was 100 cm.

^{60}Co gamma rays: A 9,000 Ci ^{60}Co gamma ray source was used in these experiments. The distance from the source to the midline of each animal was 256 cm. The midline exposure rate was 20 R/min in air for both sources.

During exposure the animals were individually housed in Lucite boxes which were so arranged that each received equal unilateral exposure. All animals received a single whole-body exposure of 1200 R.

Following removal of the animals from the exposure room, three rats from each experimental group as well as from the sham irradiated controls were sacrificed by decapitation at 0, 1, 2, 4, 6, or 24 hours postirradiation. The livers were immediately excised, chilled in an ice-cold isotonic solution of sodium chloride, and homogenized in a medium containing phosphate buffer, sucrose, nicotinamide and magnesium chloride. The exact composition of the medium and the conditions of homogenization are described elsewhere.³

Cell-free homogenates containing the fatty acid synthesizing enzyme system were then prepared and incubated at 37°C in the presence of nicotinamide adenine dinucleotide (NAD), citrate and ^{14}C -acetate as substrate. The synthesized fatty acids were chemically isolated and their radioactivity was determined in a gas flow Geiger-Mueller counter.³ The amount of radioactivity found in the isolated fatty acids, which is a measure of the activity of the enzyme system under investigation, was calculated as counts per minute per milligram of fatty acids and termed as relative isotope concentration (RIC). The data which were obtained from the control animals were set at 100 for comparison purposes and the other values were compared with the normalized control values.

Since glucose breakdown products are utilized in the biosynthesis of fatty acids, an increased blood glucose level could result in increased lipid production by the liver enzymes catalyzing fatty acid synthesis. Ionizing radiation could cause an increase in the glucose level by stimulating an increased release of pituitary and adrenal cortical hormones.

In order to test for this possibility, blood glucose determinations were carried out using new animals. Five groups, consisting of four rats each, were used in three experiments. Group 1 was fed up to approximately 4 hours before the experiment and Group 2 was deprived of food for 24 hours. The x irradiated rats as well as those which were exposed to ^{60}Co gamma rays (Groups 3 and 4) were fed up to approximately 3 hours before irradiation. No food was given to these animals following irradiation. The animals of Group 5 were made diabetic with alloxan by injecting the drug intravenously as a 1 percent solution in isotonic saline at a dose of 40 mg per kg. In these animals the glucose determinations were carried out in blood samples drawn 72 hours after injection of the alloxan solution. The Somogyi method as modified by Nelson⁹ was used for these analyses.

An additional series of experiments designed to localize the intracellular site of the radiation effect on the fatty acid synthesizing enzyme system was performed. Cell-free liver homogenates from irradiated and control rats were fractionated by centrifugation at $10,000 \times g$ for 20 minutes, the clear supernatant solutions were decanted and the sedimented particulate fractions, which consisted mainly of mitochondria, were washed once with 10 ml of homogenization liquid per fraction and recentrifuged. Six controls and six irradiated rats were used in each of two experiments. One-half of the rats were exposed to x rays, and the remainder to gamma rays.

In order to determine whether microsomes are to any extent responsible for the changes in activity which have been observed, mitochondria-free supernatants from another set of 12 irradiated and 12 control rats were further fractionated by centrifugation at $100,000 \times g$ for 60 minutes to remove the microsomes.

These last two series of experiments were carried out with animals which were sacrificed 24 hours after irradiation.

III. RESULTS

Figure 1 shows the results which were obtained when the animals were exposed to 1200 R of x rays. The activity of the fatty acid synthesizing enzyme system for both fed and starved animals was greatly enhanced as a result of this irradiation. In the case of the fed animals which were sacrificed 24 hours after removal from the exposure room, this enhancement reached a twelvefold level over the controls. The results obtained with animals exposed to ^{60}Co gamma rays under the same conditions are shown in Figure 2. It can be seen in this figure that the effect of gamma rays was similar to that of x rays although the enhancement of the enzyme activity was less pronounced. However, a comparison of Figure 1 and Figure 2 shows that in the case of x rays the enhancement of the activity started after the first hour postirradiation whereas in the animals which were exposed to gamma rays there was a lag of approximately 4 hours.

The results of the blood glucose analyses are presented in Table I. It is shown in this table that the blood glucose was about 15 percent greater in the irradiated animals than in the controls. It is also shown that although the alloxan diabetic rats had about a 400 percent increase in blood glucose level, the activity of the enzyme system

under study, as measured by the acetate incorporation into fatty acids, was enhanced by only about 40 percent.

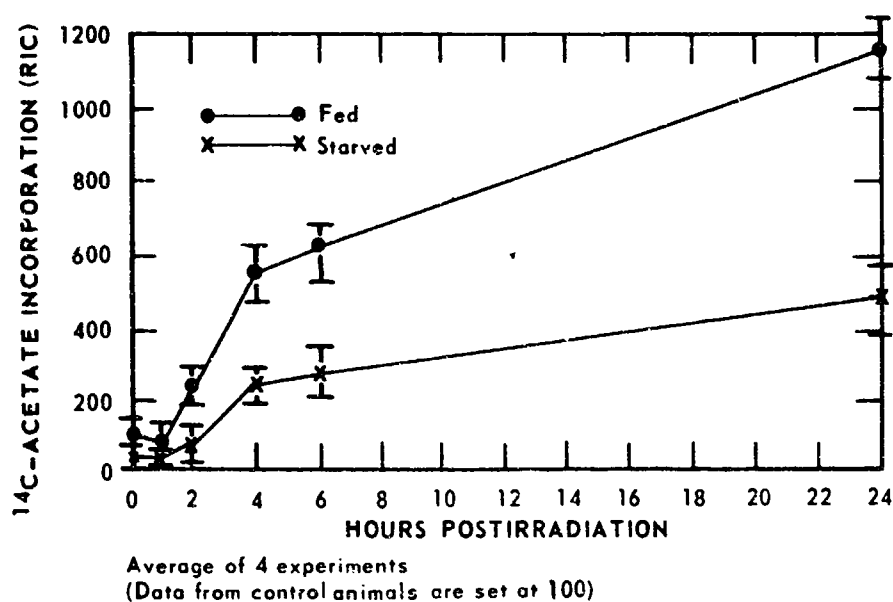


Figure 1. Fatty acid synthesis by liver enzymes in x irradiated rats (1200 R, 20 R/min)

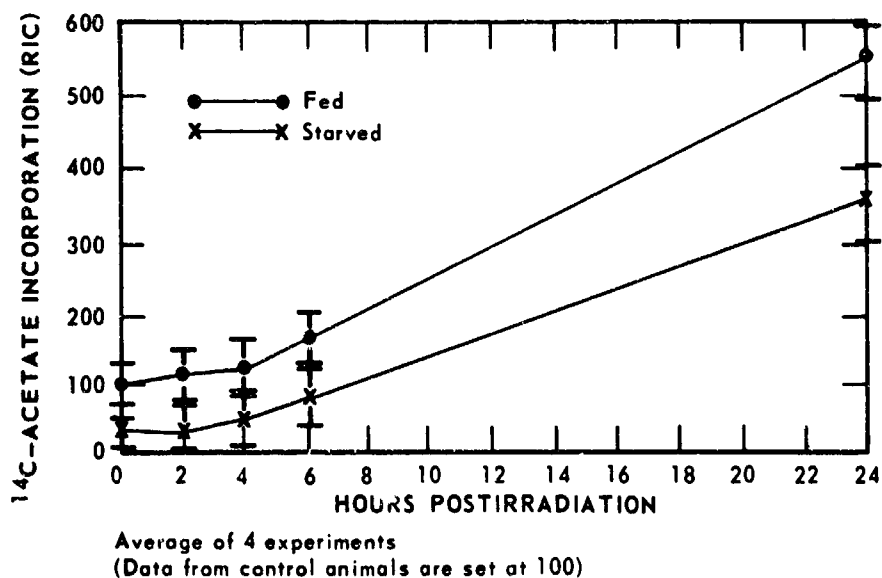


Figure 2. Fatty acid synthesis by liver enzymes in irradiated rats (^{60}Co gamma rays, 1200 R, 20 R/min)

Table I. Effect of Condition of Animal on Fatty Acid Synthesis by Liver Homogenates

Condition of rats	Glucose mg/100 ml blood	RIC*
Fed	72	100
Starved	65	23
Alloxan diabetic	336	141
X irradiated [†]	83	1150
⁶⁰ Co gamma irradiated [†]	79	540

Average of 3 experiments

* Relative Isotope Concentration (¹⁴C-acetate incorporation into fatty acids)

[†] Measured 24 hours after whole-body irradiation (1200 R, 20 R/min)

The activities of the sedimented particulate fractions alone and after recombination with the supernatants are given in Table II. It can be seen in this table that mitochondrial fractions and supernatant fluids alone from liver homogenates of control rats incorporated only small quantities of acetate carbon, and that exposure of the animals to x rays or ⁶⁰Co gamma rays increased this incorporation.

In the recombination experiments, both mitochondria and supernatant fluids from homogenates of irradiated rats increased the rate of fatty acid synthesis when added to the supernatant and mitochondrial fractions from control rats, respectively.

The results of the experiments with the microsome-free supernatants are shown in Table III. Only x irradiated animals were used in these experiments. It is shown in this table that microsomes alone, whether from x irradiated or from control rats, have negligible activity and that their removal from the supernatants or from the recombination mixtures does not cause any noticeable change in the enzymic activity.

Table II. Fatty Acid Synthesis by Liver Fractions from Control (fed) and Irradiated Rats (1200 R, 20 R/min)

	¹⁴ C-acetate incorporation (RIC*)	
	X rays	⁶⁰ Co gamma rays
Mitochondria control + Supernatant control	100	100
Mitochondria control + Supernatant irradiated	420	345
Mitochondria irradiated + Supernatant control	875	590
Mitochondria irradiated + Supernatant irradiated	588	405
Mitochondria control	3	2
Mitochondria irradiated	11	14
Supernatant control	64	37
Supernatant irradiated	305	181

Average of 2 experiments

* Relative Isotope Concentration

Table III. Fatty Acid Synthesis by Liver Fractions from Control (fed) and Irradiated Rats (x rays, 1200 R, 20 R/min)

	¹⁴ C-acetate incorporation (RIC*)
Mitochondria control + 100,000 x g Supernatant control	100
Mitochondria control + 100,000 x g Supernatant irradiated	180
Mitochondria irradiated + 100,000 x g Supernatant control	1420
Mitochondria irradiated + 100,000 x g Supernatant irradiated	1100
Mitochondria control	11
Mitochondria irradiated	8
100,000 x g Supernatant control	84
100,000 x g Supernatant irradiated	490
100,000 x g Precipitate control (microsomes)	3
100,000 x g Precipitate irradiated (microsomes)	7

Average of 2 experiments

* Relative Isotope Concentration

IV. DISCUSSION

It has been reported⁴ that x irradiation affects the hepatic incorporation of acetate into fatty acids and that, in rats fasted 24 hours prior to irradiation, an increase of incorporation was observed which reached a level nearly comparable to that of fed nonirradiated animals. Fragmentation of liver mitochondria in mice which were exposed to x irradiation has also been reported.⁸

Using liver homogenates, Catravas and Anker³ found that intact mitochondria were required for maximal acetate incorporation and that, except for nicotinamide adenine dinucleotide (NAD) and citrate, no other coenzymes or substrates were necessary.

The effect of x rays and of ⁶⁰Co gamma rays on the fatty acid synthesizing liver enzyme system has been investigated in this study and it was found that both types of irradiation greatly stimulate its activity as measured by the in vitro incorporation of ¹⁴C-acetate into fatty acids by liver homogenates.

In the case of animals which were starved 24 hours prior to irradiation, in contrast to the findings of other investigators,⁴ the enhancement of enzymic activity was found to be well above the level of fed controls and in some experiments a more than fivefold stimulation was observed.

Blood glucose analyses showed that the increase of the blood glucose level as a result of the irradiation was no more than 20 percent over the controls. It seemed to be unlikely that this increase could account for the more than twelvefold enhancement observed, for, while the blood glucose level in alloxan-diabetic animals increased by

400 to 500 percent above normal, the activity of the enzyme system under study was enhanced by only 50 to 60 percent.

Mitochondria and supernatant fluids from liver homogenates of control or irradiated rats were relatively inactive. It should, however, be mentioned that an enhancement of the activity of the soluble enzymes present in the irradiated supernatants has been observed. In the recombination experiments, both mitochondria and supernatant fluids from liver homogenates of irradiated rats increased the rate of fatty acid synthesis when added to the supernatants and mitochondria, respectively, from control rats. This finding indicates that the activities of both enzyme systems, i. e., mitochondrial and the soluble, are affected by exposure of the animal to irradiation.

The fact that the enzymic activities remain in the particle-free supernatants when microsomes are removed from the supernatant fluids shows that under our experimental conditions microsomes do not participate in the radiation-induced activity changes of the enzymic system under study.

At present, it is not clear that this increase in enzymic activity is due to net enzyme synthesis. It may be due to destruction of an inhibitor or to conformational changes in the enzyme molecule. Aggregation or disaggregation of the molecule, especially in acetyl coenzyme A carboxylase, the branch point enzyme in fatty acid synthesis, could easily account for part of the activity changes observed in this study. The activation of this enzyme by intermediates of the tricarboxylic acid cycle is accompanied by increase of its sedimentation constant¹⁶ and polymerization of its subunits.¹⁰

One also should not exclude the possibility of leakage through the mitochondrial membrane, the chemical structure of which could be altered by ionizing radiation. Research is in progress to verify these hypotheses.

V. SUMMARY

It has been shown in this study that the activity of the liver enzyme system which is responsible for the biosynthesis of fatty acids is greatly enhanced by x or ^{60}Co gamma radiation whether the animal was fed or fasted prior to irradiation and that both mitochondrial and soluble enzymes are affected by radiation, the latter to a much greater extent.

Blood glucose determinations which were carried out in control, irradiated, and alloxan-diabetic rats indicate that the enhancement observed is not due to an increase of the blood glucose level of the animal as a result of the radiation exposure but to some other factor the nature of which has not yet been elucidated.

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